# Clinical review

*Science, medicine, and the future*

## Motor neurone disease

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Motor neurone disease is one of the most common neurodegenerative diseases of adult onset. Currently, there is no treatment that substantially slows disease progression, and average survival from the start of symptoms is only about three years. As this article discusses, however, new understanding of disease pathogenesis is suggesting the way toward more effective neuroprotective treatments aimed at slowing or arresting injury to motor neurones.

### Future developments

Identification of further genetic mutations in familial motor neurone disease and of genetic factors associated with sporadic disease

Use of cellular and animal models of motor neurone disease to identify the sequential molecular events leading to death of motor neurones and to evaluate new neuroprotective treatments

New treatment for patients with a "cocktail" of neuroprotective compounds and better supportive care

As scientific developments prolong survival, attention will become focused on quality of life

### Background

The incidence of the disease is one to two per 100 000, and a general practice with 10 000 patients is likely to encounter a case of motor neurone disease every two to three years. The disease predominantly affects middle aged and elderly people, with a mean age of onset of 55 years, although younger people are occasionally affected. The disease is sporadic in 90% of cases, but about 10% are familial, usually with an autosomal dominant mode of inheritance.

The disease causes progressive injury and cell death of lower motor neurone groups in the spinal cord and brain stem and usually also of upper motor neurones in the motor cortex. Those affected typically develop a combination of upper and lower motor neurone signs, with progressive muscle weakness and wasting usually accompanied by pathologically brisk reflexes, eventually involving the limb and bulbar muscles. Clinical variants of the disease may affect purely the lower motor neurones (progressive muscular atrophy) or the upper motor neurones (primary lateral sclerosis). Death usually results from respiratory failure due to weakness of the ventilatory muscles.

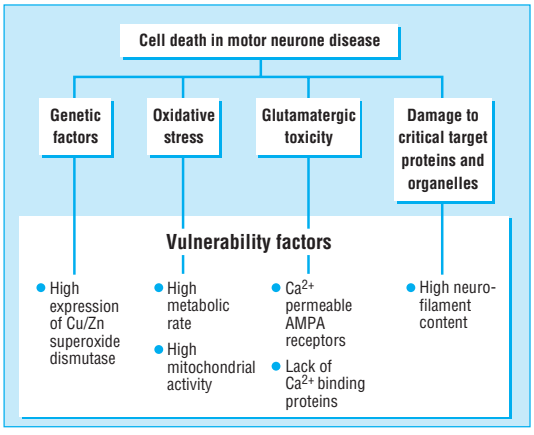


**Fig 1** Major factors that seem to be contributing to motor neurone injury and the cell specific features of motor neurones that may render these cells vulnerable to such insults

The precise causes of the neurodegenerative process remain unknown. The selectivity of the disease process for the motor system is now recognised to be relative rather than absolute. Careful clinical and pathological studies have revealed that extra-motor parts of the central nervous system are also affected. Thus, motor neurone disease is now regarded as a multisystem disease in which the motor neurones tend to be affected earliest and most severely. Scientific advances are starting to yield insights into the mechanisms of motor neurone degeneration and into the specific features of motor neurones that may render these cells vulnerable to pathology (fig 1).

### Pathogenesis of motor neurone disease

#### Genetics

Multiple different abnormal gene products can set the scene for motor neurone degeneration. More than 60 different mutations, mainly point mutations, have been described in about 250 pedigrees of familial motor neurone disease. One of the most important findings

has been that mutations in the gene on chromosome 21 encoding the enzyme Cu/Zn superoxide dismutase ($SOD_1$) underlie 20% of familial cases of motor neurone disease and 2% of all cases.[1] The primary function of this enzyme is to catalyse the conversion of intracellular superoxide radicals produced during normal cellular metabolism to hydrogen peroxide, which is then eliminated by other free radical scavenging enzymes. It is a ubiquitous enzyme, expressed in cells throughout the body, and why motor neurones should be especially vulnerable to injury in the presence of mutations in the enzyme is not clear. However, motor neurones have a high expression of Cu/Zn superoxide dismutase, in both the cell body and axonal compartments, compared with other cells in the nervous system.

Evidence suggests that the mutant enzyme exerts its deleterious effects, not through a loss of its normal function, but through a toxic gain of function. Various hypotheses, for which there is some experimental support, have been put forward to explain this.[2,3] The most favoured is that the mutant enzyme may handle biochemical compounds such as hydrogen peroxide and peroxynitrite abnormally, resulting in increased formation of highly damaging hydroxyl radicals and formation of nitrotyrosine residues on intracellular proteins respectively. There have also been suggestions that mutant Cu/Zn superoxide dismutase may form abnormal intracellular aggregates, with an adverse effect on cellular biochemistry, or that there may be abnormal cytosolic release of copper or zinc, with resulting neurotoxicity.

The genetic alterations underlying the remaining 80% of familial cases of motor neurone disease are still unknown. Linkage analysis has revealed that chromosomes 2q and 9q may harbour genetic alterations underlying rare forms of motor neurone disease of juvenile onset.[4,5] Isolated reports have described alterations in the gene encoding the neurofilament heavy protein, an important component of the cytoskeleton of neurones[6,7]; in subunit 1 of the cytochrome c oxidase subunit, part of the mitochondrial respiratory chain[8]; and in abasic (AP) endonuclease, a key enzyme in the repair of oxidative damage to DNA.[9]

## Oxidative stress

The effects of oxidative stress within non-replicating cells such as neurones may be cumulative, and injury by free radical species is a major potential cause of the age related deterioration in neuronal function occurring in several neurodegenerative diseases. There is particularly keen interest in the role of oxidative stress in motor neurone disease given that mutations in the gene for Cu/Zn superoxide dismutase underlie some cases of familial disease. Studies of human postmortem tissue from the central nervous system have demonstrated the presence of biochemical changes to proteins and DNA that represent "footprints" of free radical damage, and these changes are more pronounced in cases of motor neurone disease compared with controls.[10,11] Other postmortem neurochemical changes, including altered expression of components of the intracellular antioxidant defence systems, have been interpreted to indicate an attempted compensatory response to the presence of oxidative stress during the course of motor neurone disease.[12] Fibroblasts cultured from the skin of patients with both familial and sporadic motor neurone disease show increased sensitivity to oxidative insults compared with those from controls.[13]

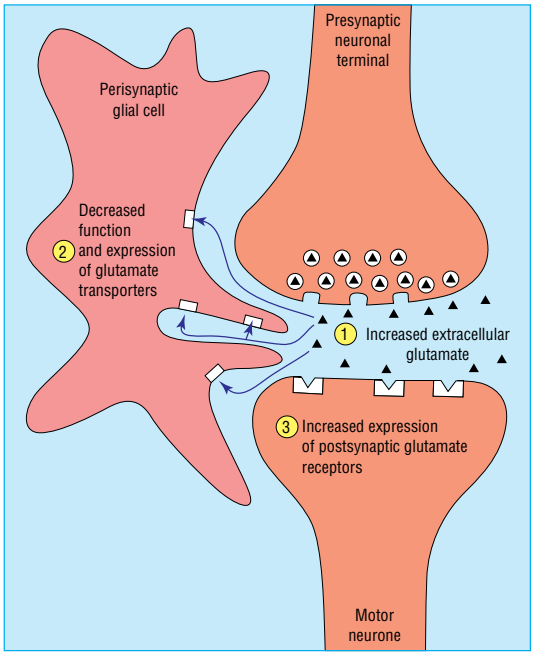


**Fig 2** Evidence for alteration in the glutamate neurotransmitter system in motor neurone disease

## Excitotoxicity

Glutamate is the main excitatory neurotransmitter in the human nervous system. The excitatory signal is terminated by removal of glutamate from the synaptic cleft by transporter proteins, which are largely located on perisynaptic glial cells (fig 2). Excessive stimulation of neuronal glutamate receptors can damage and kill these cells by mechanisms including derangement of intracellular calcium homoeostasis and production of free radicals—the term excitotoxicity has been coined for this process.

Circumstantial evidence has implicated glutamate mediated toxicity as a contributory factor to cell injury in motor neurone disease.[14] The key findings are that the expression and function of excitatory amino acid transporter 2 (EAAT2), the major glutamate reuptake transporter protein, may be impaired in motor neurone disease and that concentrations of glutamate may be abnormal in extracellular and cerebrospinal fluid in at least some patients with the disease.[15–17]

Recently, the presence of abnormally spliced RNA transcripts for excitatory amino acid transporter 2 have been uncovered in the motor system of patients with motor neurone disease, and it has been suggested that a specific defect of RNA processing may be an important aetiological factor in the disease.[18] However, others have suggested that these alternatively spliced RNA transcripts may be found in the normal central nervous system.[19]

## Vulnerability of motor neurones to neurodegeneration

Motor neurones are fixed, post-mitotic cells that are no longer capable of replication and which gradually

decline in number with age. A tantalising feature of motor neurone disease is why only specific populations of motor neurones are susceptible to the disease process. Some populations of cells, including those controlling the extraocular muscles and the pelvic floor, remain relatively unscathed during the disease.

Some progress has been made in identifying the features of motor neurones that may render them vulnerable.[20] Firstly, motor neurones are one of the largest cells in the nervous system, and those supplying distal lower limb muscles may have to support axonal processes up to one metre long. This leads to high energy demands, and thus a high level of mitochondrial activity, as well as the need for a robust cytoskeleton, which requires a high intracellular content of neurofilament proteins. There is evidence from human tissue and experimental models that mitochondria and neurofilament proteins may be particular targets for injury within motor neurones.[2]

Secondly, motor neurones may have particular cellular features which render them vulnerable to calcium mediated toxic processes after activation of their cell surface glutamate receptors. Motor neurones seem to have a specific profile of calcium permeable glutamate receptors that is different from that of many other cell groups in the central nervous system.[21] In addition, motor neurones lack certain intracellular calcium buffering proteins that may protect neurones from excessive increases in cytosolic calcium.[22]

Motor neurones also have a high perisomatic expression of the excitatory amino acid transporter 2 and a very high intracellular expression of Cu/Zn superoxide dismutase. These proteins may help to protect normal motor neurones from the effects of glutamate toxicity and oxidative stress respectively, but their high level of expression may render motor neurones vulnerable in the face of genetic or post-translational alterations interfering with the function of these proteins.

**Potential treatments for motor neurone disease that have undergone recent trials**

**Clinical trials in patients**

*Antiglutamate agents*

- Riluzole (positive effect)
- Lamotrigine (negative effect)
- Gabapentin (non-significant positive effect)

*Antioxidant agents*

- *N*-acetylcysteine (non-significant positive effect)

*Neurotrophic factors*

- Ciliary neurotrophic factor (negative effect)
- Brain derived neurotrophic factor (negative effect when give subcutaneously)
- Insulin-like growth factor 1 (positive effect in one trial, not replicated in second study)

**Drugs showing neuroprotective effects in experimental models**

*Cellular models*

- Glutathione
- Vitamin E
- Mimics of Cu/Zn superoxide dismutase
- Copper chelators
- Caspase inhibitors

*Transgenic mice models*

- Vitamin E
- Riluzole
- Gabapentin
- Penicillamine
- Overexpression of Bcl-2
- Inhibition of interleukin 1 β converting enzyme

### Experimental models

Cultured neuronal cells manipulated to express mutant genes for human Cu/Zn superoxide dismutase show increased mortality when exposed to conditions of oxidative stress compared with neurones expressing normal Cu/Zn superoxide dismutase.[2] In addition, the mutant enzyme seems to have an altered affinity for hydrogen peroxide, leading to increased formation of highly toxic hydroxyl radicals. There is also evidence that intracellular calcium homoeostasis may be impaired in the presence of the mutant enzyme.

Three mutant Cu/Zn superoxide dismutase transgenic mouse strains have been developed which develop a motor neurone disease phenotype similar to the human disease. Potentially important pathophysiological changes observed in this mutant mouse model include impaired transport function within the axons of motor neurones, increased calcium concentrations in motor neuronal terminals, and early pathological changes affecting the mitochondria of motor neurones which precede clinical manifestations of the disease.[2]

## Treatment

### Human clinical trials

Several therapeutic agents have recently been tested in clinical trials in patients with motor neurone disease (see box). Riluzole is a sodium channel blocker that inhibits the release of glutamate and has several other potentially neuroprotective effects: it had a modest effect in prolonging survival.[23] There have been no well powered trials of antioxidant compounds in motor neurone disease: a small scale trial using *N*-acetylcysteine found a non-significant improvement in survival in patients whose disease symptoms started in the limb muscles.[24]

Several neurotrophic factors have recently been evaluated in clinical trials. These compounds can protect motor neurones from injury via several mechanisms, and many trophic factors from different gene families have been shown to promote survival of motor neurones in vivo and in vitro. However, only one neurotrophic factor, insulin-like growth factor 1, has produced a positive effect in slowing disease progression,[25] but this result was not replicated in a second trial. There is concern about the route of administration of neurotrophic factors: to date, they have been administered subcutaneously, and it is possible that they are not reaching motor neurones in sufficient amounts to exert a maximal effect. Direct intrathecal administration via an implanted infusion pump is one possible solution.

### Experimental models

One antioxidant compound, vitamin E, and two antiglutamate agents, riluzole and gabapentin, have shown beneficial effects in transgenic mice with a mutant Cu/Zn superoxide dismutase enzyme.[26] Vitamin E delayed onset of motor neurone disease, whereas antiglutamate therapy extended survival once the disease became manifest. These findings suggest that oxidative stress may contribute to the early stages of motor neurone injury, with glutamate toxicity playing a part later in the cell death cascade. The effects of changes in gene expression on murine motor neurone disease have been investigated by cross breeding mice with mutant superoxide dismutase with other transgenic

strains. For example, the onset of motor neurone degeneration in mice with the mutant enzyme was slowed by concomitant overexpression of Bcl-2, a protein that inhibits apoptosis (programmed cell death).[27]

In cellular models of motor neurone disease, agents showing a positive neuroprotective action include vitamin E, increased expression of Bcl-2, inhibition of caspases (proteolytic enzymes activated during apoptosis), compounds mimicking Cu/Zn superoxide dismutase, the antioxidant glutathione, and copper chelators.[28]

## Future directions

Cell injury in motor neurone disease seems to reflect a complex interplay between genetic factors, oxidative stress, and imbalance of the glutamatergic excitatory control of motor neurones, which may result in damage to critical target proteins and organelles. The relative importance of these factors is likely to vary in different subgroups of patients.

Observations in animal and cellular models are likely to clarify our knowledge of the cellular mechanisms of motor neurone disease over the next few years. Priorities for research include the search for other genes associated with familial motor neurone disease, for genetic factors predisposing to the sporadic form of the disease, and for insights into the cell specific biochemistry and physiology of motor neurones. These strategies are likely to lead to the development of more effective neuroprotective treatment for patients. Future treatment is likely to involve a "cocktail" of neuroprotective compounds interfering with several neuronal injury pathways.

In using treatments aimed at retarding or arresting motor neurone injury, we will need to pay close attention to patients' quality of life. Thus, if neuroprotective treatment prolongs patients' survival, we must ensure that they consider the quality of this extra life to be acceptable.

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1 Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59-62.
2 Cookson MR, Shaw PJ. Oxidative stress and motor neurone disease. *Brain Pathol* (in press).
3 Brown RH. Amyotrophic lateral sclerosis: recent insights from genetics and transgenic mice. *Cell* 1995;80:687-92.
4 Hentati A, Bejaoui K, Pericak-Vance M, Hentati F, Speer MC, Hung WY, et al. Linkage of recessive familial amyotrophic lateral sclerosis to chromosome 2q33-q35. *Nat Genet* 1994;7:425-8.
5 Chance PF, Rabin BA, Ryan SG, Ding Y, Scavina M, Crain B, et al. Linkage of the gene for an autosomal dominant form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. *Am J Hum Genet* 1998;62:633-40.
6 Figlewicz DA, Rouleau GA, Krizus A, Julien JP. Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. *Hum Mol Genet* 1994;3:1757-61.
7 Tomkins J, Usher PA, Slade JY, Ince PG, Curtis A, Bushby K, et al. Novel insertion in the KSP repeat region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS). *Neuroreport* (in press).
8 Comi GP, Bordoni A, Salani S, Franceschina L, Scianno M, Prelle A, et al. Cytochrome c oxidase subunit 1 microdeletion in a patient with motor neuron disease. *Ann Neurol* 1998;43:110-6.
9 Olkowski ZL. Mutant AP endonuclease in patients with amyotrophic lateral sclerosis. *Neuroreport* 1998;9:239-42.
10 Ferrante RJ, Brown SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U, et al. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J Neurochem* 1997;69:2064-74.
11 Shaw PJ, Ince PG, Falkous G, Mantle D. Oxidative damage to protein in sporadic motor neuron disease spinal cord. *Ann Neurol* 1995;38:691-5.
12 Shaw PJ, Chinnery RM, Thagesen H, Borthwick G, Ince PG. Immunocytochemical study of the distribution of the free radical scavenging enzymes Cu/Zn superoxide dismutase (SOD1), Mn superoxide dismutase (Mn SOD) and catalase in the normal human spinal cord and in motor neurone disease. *J Neurol Sci* 1997;147:115-25.
13 Aguirre T, Van den Bosch L, Goetschalckx K, Tilkin P, Mathijis G, Cassiman JJ, et al. Increased sensitivity of fibroblasts from amyotrophic lateral sclerosis patients to oxidative stress. *Ann Neurol* 1998;43:452-7.
14 Shaw PJ, Ince PG. Glutamate, excitotoxicity and amyotrophic lateral sclerosis. *J Neurol* 1997;244(suppl 2):S3-14.
15 Rothstein JD, Martin LJ, Kuncl RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N Engl J Med* 1992;326:1464-8.
16 Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 1995;38:73-84.
17 Shaw PJ, Forrest V, Ince PG, Richardson JP, Wastell HJ. CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration* 1995;4:209-16.
18 Lin CLG, Bristol LA, Dykes-Hoberg M, Crawford T, Clawson L, Rothstein JD. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter in amyotrophic lateral sclerosis. *Neuron* 1998;20:589-602.
19 Nagai M, Abe K, Okamoto K, Itoyama Y. Identification of alternative splicing forms of GLT-1 mRNA in the spinal cord of amyotrophic lateral sclerosis patients. *Neurosci Lett* 1998;244:165-8.
20 Shaw PJ, Eggett CJ. Molecular factors underlying the selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. *J Neurol* (in press).
21 Williams TL, Day NC, Ince PG, Kamboj RK, Shaw PJ. Calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors: a molecular determinant of selective vulnerability in amyotrophic lateral sclerosis. *Ann Neurol* 1997;42:200-7.
22 Ince PG, Stout N, Shaw PJ, Slade J, Hunziker W, Heizmann CW, et al. Parvalbumin and calbindin D-28k in the human motor system and in motor neuron disease. *Neuropathol Appl Neurobiol* 1993;19:291-9.
23 Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V, ALS/riluzole Study Group II. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. *Lancet* 1996;347:1425-32.
24 Louwerse ES, Weverling GJ, Bossuyt PMM, Posthumus Meyjes FE, de Jong JMBV. Randomized, double-blind controlled trial of acetylcysteine in amyotrophic lateral sclerosis. *Arch Neurol* 1995;52:559-64.
25 Lai EC, Felice KJ, Festoff BW, Gawel MJ, Gelinas DF, Kratz R, et al. Effect of recombinant human insulin-like growth factor-1 on progression in ALS. *Neurology* 1997;49:1621-30.
26 Gurney ME, Cutting FB, Zhai P, Doble A, Taylor CP, Andrus PK, et al. Benefit of vitamin E, riluzole and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann Neurol* 1996;39:147-58.
27 Kostic V, Jackson-Lewis V, deBilbao F, Dubois-Dauphin M, Przedborski S. Bcl-2: prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Science* 1997;277:559-62.
28 Ghadge GD, Lee JP, Bindokas VP, Jordan J, Ma L, Miller RJ, et al. Mutant superoxide dismutase-1-linked familial amyotrophic lateral sclerosis: molecular mechanisms of neuronal death and protection. *J Neurosci* 1997;17:8756-66.

## Give a remedy a thorough trial

I dislike much the habit that many young practitioners form, namely, that of vaunting some one article of medicine as a specific for a certain disease. *Specifics, we have none*, and it but brings the profession into disrepute when some foolish ecstatic throws chaff (when we expect sound wheat) which, as it flies, is caught up and devoutly hugged by a certain class who try the remedy because it is new, and has been lauded by its crack-brained originator, but to fail miserably and lose the confidence of their patients, which in truth their ability never entitled them to. The poor patient who falls into their unmerciful hands, has been endowed by nature with a Herculean frame, and despite the rigor of the disease and the poisonous efforts of the so-called *specific*, he struggles through, a miserable wreck, escaping with barely life, while our scarce fledged doctor seizes his goose-quill and flings to the world through the pages of some medical journal, his new found specific for a disease which *nature* conquered in spite of the stumbling blocks he placed in her way. Give a remedy a thorough trial before you speak of it as being adapted for the cure of a disease.

Ussher BB. *The Chicago Medical Times* 1869;1:116.

Submitted by J H Baron, *honorary professorial lecturer, New York*